Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplement/Appendix

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2.1 Protocol Modification, June 2015

In May, 2015, it became clear that the proportion of Eos low vs Eos high would not be 1:1, as we had projected based on prior published data. Rather, after the first 101 participants had been randomized, the distribution of Eos low:Eos high was 78:23 (~3.4:1).

After a thorough analysis of procedures and protocols for sputum induction and processing, and recount of 100% of samples, the Steering Committee modified the statistical analysis plan to revise the order of our study objectives to more directly address the overarching research question: "Among patients with asthma who are persistently non-eosinophilic (<2% sputum eosinophils in two induced sputum samples collected 3-6 weeks apart), is there a preference for inhaled corticosteroids (ICS) or long-acting muscarinic antagonist (LMA) compared to placebo?" Rather than maintaining the **primary analysis** as a comparison between the non-eosinophilic and eosinophilic groups, we focused the primary comparison on ICS vs PBO and LMA vs PBO within the non- eosinophilic group. The study design, enrollment, and randomization procedures did not change. The primary outcome remained a composite based on the three components of treatment failure, asthma control days (ACDs), and FEV_1. For each SIENA participant, we compared ICS to placebo and LMA to placebo in a hierarchical manner. For the primary analysis, this comparison was done in the non-eosinophilic group only. The comparison between the non-eosinophilic and eosinophilic strata, as described in the original protocol, became a secondary analysis.

In order to describe the primary and secondary null hypotheses, we introduced the following notation:

- $p_{Eos–,ICS>Placebo}$ = probability that ICS is superior to placebo within the non-eosinophilic phenotype
- $p_{Eos–,Placebo>ICS}$ = probability that placebo is superior to ICS within the non-eosinophilic phenotype
- $p_{Eos–,ICS\approx Placebo}$ = probability that ICS and placebo are equivalent within the non-eosinophilic phenotype = $1 – p_{Eos–,ICS>Placebo} – p_{Eos–,Placebo>ICS}$
- $p_{Eos–,LMA>Placebo}$ = probability that LMA is superior to placebo within the non-eosinophilic phenotype
- $p_{Eos–,Placebo>LMA}$ = probability that placebo is superior to LMA within the non-eosinophilic phenotype
- $p_{Eos–,LMA\approx Placebo}$ = probability that LMA and placebo are equivalent within the non-eosinophilic phenotype = $1 – p_{Eos–,LMA>Placebo} – p_{Eos–,Placebo>LMA}$

For probabilities within the eosinophilic phenotype, we simply changed "Eos–" to "Eos+" in the subscript notation.

The co-primary research hypotheses were that among differential responders in the non-eosinophilic phenotype, ICS is superior to placebo and LMA is superior to placebo. In statistical terms, the null hypotheses are:

(1) H_0: $p_{Eos–,ICS>Placebo} = p_{Eos–,Placebo>ICS}$

(2) H_0: $p_{Eos–,LMA>Placebo} = p_{Eos–,Placebo>LMA}$

We will apply two-sided, exact binomial tests at the 0.025 significance level (Bonferroni correction) for each of these null hypotheses. We will also fit logistic regression models to the hierarchical composite outcomes to allow adjustment for period differences and seasonal effects as a sensitivity analysis.

Secondary analyses with the primary outcome will include the following:

- Comparisons within the eosinophilic phenotype in terms of the null hypotheses
    1. H_0: $p_{Eos+,ICS>Placebo} = p_{Eos+,Placebo>ICS}$
    2. H_0: $p_{Eos+,LMA>Placebo} = p_{Eos+,Placebo>LMA}$.
- Comparisons between the non-eosinophilic and eosinophilic phenotypes in terms of the null hypotheses
    1. H_0: $p_{Eos–,ICS>Placebo} = p_{Eos+,ICS>Placebo}$
    2. H_0: $p_{Eos–,LMA>Placebo} = p_{Eos+,LMA>Placebo}$.
- Comparison of the ICS and LMA treatments in the manner described above for the primary and secondary analyses (within the non-eosinophilic phenotype, within the eosinophilic phenotype, and between the eosinophilic and non-eosinophilic phenotypes, respectively).
    1. H_0: $p_{Eos–,ICS>LMA} = p_{Eos-,LMA>ICS}$
    2. H_0: $p_{Eos+,ICS>LMA} = p_{Eos+,LMA>ICS}$
    3. H_0: $p_{Eos–,ICS>LMA} = p_{Eos+,ICS>LMA}$

Power: Based on this new analysis plan, the new target sample size for the SIENA trial was 336 randomized participants, 262 in the non-eosinophilic phenotype and 74 in the eosinophilic phenotype. For the purpose of this calculation, we assumed (conservatively) a ratio of non-eosinophilic:eosinophilic of 3.5:1.

For the co-primary comparisons within the non-eosinophilic phenotype, the sample size of 262 yielded statistical power of 0.9 with two-sided, 0.025 significance level tests (Bonferroni correction), while allowing for a 15% drop-out rate, to detect a difference in probabilities of 0.20. We assumed that 30% of the participants would not display a preference for ICS versus placebo (and that 30% of the participants would not display a preference for LMA versus placebo). The following table illustrates the level of statistical power for selected sample sizes.

Number Randomized	Number of Completers	Number of Differential Responders	$p_{Eos-,Trt \approx Placebo}$	Difference in Probabilities	Statistical Power
202	172	120	0.30	0.20	0.80
228	194	136	0.30	0.20	0.85
262	223	156	0.30	0.20	0.90

With respect to the secondary analysis of the primary outcome, the following table illustrates the statistical power for detecting $p_{Eos+,ICS>Placebo} = 0.71$ and $p_{Eos-,ICS>Placebo} = 0.45$, yielding a difference of 0.26 between the two phenotypes (and for detecting $p_{Eos+,LMA>Placebo} = 0.71$ and $p_{Eos-,LMA>Placebo} = 0.45$, yielding a difference of 0.26 between the two phenotypes).

EOS-Negatives		EOS-Positives		Statistical Power
N	$p_{Eos-,ICS>Placebo}$ or $p_{Eos-,LMA>Placebo}$	N	$p_{Eos+,ICS>Placebo}$ or $p_{Eos+,LMA>Placebo}$	
202	0.45	58	0.71	0.80
228	0.45	66	0.71	0.85
262	0.45	74	0.71	0.90

For the secondary analysis of comparing the ICS and LMA treatments within the non-eosinophilic phenotype, there is 90% statistical power with a sample size of 262 to detect a difference of 0.185 with a two-sided, 0.05 significance level test.

3.1 Inclusion criteria for enrollment (Week 0)

All participants will meet ALL of the following inclusion criteria:

1. Males or females age 12 or greater (at week 0);
2. Physician-diagnosed asthma or a history consistent with asthma for at least previous 12 months (at week 0);
3. Asthma confirmed by:
   (a) β-agonist reversibility of FEV1 ≥12% and ≥ 200ml following 4 puffs albuterol (at week 0) OR
   (b) methacholine PC20 ≤ 16 mg/ml (at visit 1A). Source documentation for PC20 from an AsthmaNet methacholine challenge completed within 6 months of week 0 will be accepted;
4. No use of oral corticosteroid for at least 6 weeks or inhaled corticosteroid for at least 3 weeks (at week 0). Individuals who are taking low-dose ICS (equivalent of BDP 80-240 mcg/day), intermittent (<5 days/week) ICS or intermittent ICS/LABA who are well controlled may be withdrawn from ICS or ICS/LABA prior to enrollment in the Run In (see *Supervised Washout*, page 36)
5. No use of leukotriene modifier for at least 3 weeks (at week 0). Individuals who are taking LTRA who are well controlled may be withdrawn from LTRA prior to enrollment in the Run In (see *Supervised Washout*, page 36)
6. Prebronchodilator FEV1 ≥ 70% of predicted (at week 0);
7. At least 1 of the following indications for chronic controller therapy:
   (a) Asthma Symptoms > 2 days/week OR
   (b) Nocturnal Asthma Symptoms > 2 nights/month OR
   (c) Short-acting beta-2 agonist use for symptom control (not prevention of EIB)> 2 days/week
8. Ability to provide screening and baseline information at week 0;
9. Ability and willingness to provide informed consent at week 0;
10. Ability to perform spirometry as per ATS criteria;
11. For women of childbearing potential: not pregnant, non-lactating, and agree to practice an adequate birth control method (abstinence, single barrier methods or combination barrier and spermicide, or hormonal) for the duration of the study (at week 0);
12. If intranasal steroids might be needed, willingness to take a single agent at a stable dose throughout the trial, starting prior to or on enrollment in the run-in period at week 0.

3.2 Exclusion criteria for enrollment (Week 0)

All participants will be excluded for ANY of the following exclusion criteria at week 0:

1. Chronic oral corticosteroid therapy; OR
2. Chronic inhaled corticosteroid therapy OR
3. New allergen immunotherapy within the past 3 months or anticipated changes to an ongoing immunotherapy regimen. Stable allergen immunotherapy for at least the past 3 months is acceptable.; OR
4. Use of omalizumab within 3 months, OR
5. History of bladder-neck obstruction, urinary retention, BPH, OR
6. History of narrow angle glaucoma, OR
7. History of significant cardiovascular disorders and arrhythmias, OR
8. History of life-threatening asthma requiring treatment with intubation or mechanical ventilation within the past 5 years; OR
9. Prebronchodilator FEV1 < 70% of predicted OR
10. Asthma exacerbation within past 6 weeks requiring systemic corticosteroids (evaluated at week 0) OR
11. Respiratory tract infection within past 4 weeks; OR
12. History of smoking (cigarettes, cigars, pipes, marijuana or any other substances) within the past 1 year, or > 10 pack-years total if ≥ 18 years of age, or > 5 pack- years total if < 18 years of age; OR
13. Chronic diseases or medical conditions (other than asthma) that in the opinion of the investigator would prevent participation in trial or put the participant at risk by participation, e.g. chronic diseases of the lung (other than asthma), heart, liver, kidney, endocrine or nervous system, or immunodeficiency; OR
14. Use of investigative drugs or enrollment in intervention trials in the 30 days prior to screening or during the study; OR
15. Use of any drug prohibited during the study or within the washout period prior to week 0; OR
16. Any condition or compliance issue which, in the opinion of the investigator, might interfere with participation in the study; OR
17. Inability or unwillingness to perform required study procedures.

3.3 Criteria for Stratification based on Sputum Eosinophils

All participants underwent sputum induction up to 3 times during the Run-in (at entry and at 3 and 6 weeks if necessary for eligibility), in order to obtain 2 acceptable sputum samples for assessment of sputum cell counts. Participants whose initial sputum sample was unacceptable based on our standard criteria (≥80% squamous cells) were asked to provide a second sample. If this was also unacceptable, they were excluded from the study.

Based on a "cut point" of ≥ 2% eosinophils and two measures of sputum eosinophil % during the run-in, participants were categorized as "eosinophilic" (either persistently or intermittently eosinophilic) or "persistently non-eosinophilic" and stratified on this basis at randomization. Those with sputum eosinophils ≥ 2% were deemed “Eos high”; those with <2% sputum eosinophils were deemed “Eos low”.

4.1 Definition of Treatment Failure

The definition of Treatment Failure is based on the Symptom-Based Action Plan that was used successfully in the ACRN IMPACT Study and includes:

- Awakening from asthma three or more times in a two-week period or on two consecutive nights, or
- Using albuterol for relief of symptoms four or more times/day for two or more consecutive days, or
- Albuterol has been relieving symptoms for less than four hours after treatment, or
- Using albuterol for relief of symptoms daily for seven days, and this use exceeds two times the weekly use of albuterol in the baseline period, or
- Exercise induces unusual breathlessness.

4.2 Definition of Asthma Exacerbation:

Although all participants with an asthma exacerbation will also meet the criteria outlined for treatment failure above, asthma exacerbations are more severe episodes of acute worsening, defined by meeting criteria for treatment failure AND one or more of the following:

- Failure to respond within 48 hours to treatment failure rescue algorithm
- FEV1 <50% of baseline on 2 consecutive measurements
- FEV1 <40% of predicted on 2 consecutive measurements
- Use of ≥ 16 puffs of "as needed" β-agonist per 24 hours for a period of 48 hours
- Experiencing an exacerbation of asthma in the opinion study investigator or personal physician
- Use of oral/parenteral corticosteroid due to asthma

5.1 Primary Outcome Measure

The primary outcome is a hierarchical composite of three measures of asthma control, assessed during the last 8 weeks of each 12 week treatment period: Treatment Failure (TF), Asthma Control Days (ACD), and FEV_1.

Definition of Treatment Failure: See **4.1** above.

ACDs were documented in daily diaries, and were defined as: A day with no rescue albuterol use (pre-exercise albuterol was not counted), no non-study asthma medications, no daytime asthma symptoms (shortness of breath, wheezing, chest tightness, phlegm/mucus rated as mild, moderate or severe, or cough rated as moderate or severe), no night time asthma symptoms, no unscheduled healthcare visits for asthma, and no PEF <80% of predetermined baseline.

FEV_1 is a standard outcome measure for asthma, and was used in a similar hierarchical preference analysis in BADGER.

5.2 Secondary and Exploratory Outcome Measures

Secondary Outcome Measures

Each of the three components of the composite outcome (TF, ACD, FEV_1) will be analyzed separately as secondary outcomes. Other secondary outcomes include PEF, asthma exacerbations, time to treatment failure and time to first exacerbation.

Exploratory Outcome Measures

An important exploratory question is whether other biomarkers such as blood periostin, blood eosinophils or eNO can be used instead of sputum eosinophils to identify patients with differential treatment preferences to ICS and LMA. Although recent data suggest that airway eosinophilia, elevated FeNO, and serum periostin may all be markers of TH2 inflammation, we chose to stratify our populations based on sputum eosinophilia, a robust biomarker that has been well-characterized. Periostin, a 90 kD protein produced by airway epithelium in response to IL-13, is an alternate candidate biomarker, but more information is needed about how blood periostin levels relate to airway eosinophil levels, and about the threshold value for defining abnormal periostin levels. FeNO is another candidate biomarker of airway eosinophilia and ICS responsiveness but two recent reports have questioned its utility as a biomarker of airway eosinophilia.[1,19] In this prospective study we collected serum for periostin and measured eNO and blood eosinophils so that we can evaluate the relative utility of these three simpler tests as biomarkers of airway eosinophilia and ICS treatment response in mild moderate asthma. We also proposed to assess the bronchodilator response (BR) to both beta agonist and anticholinergic agents to determine whether the eosinophil-negative group has different bronchodilator responses to albuterol vs ipratropium (Atrovent® HFA).

We included adolescents 12-18 years old in this study because asthma guidelines combine this group with adults, but the study was not powered for the comparison between adults and adolescents. This important exploratory analysis will provide clues as to the prevalence of eosinophil negative asthma in adolescents, the utility of and appropriate cut point for periostin, and the similarity or difference in the treatment response between adolescents and adults.

Additional exploratory outcomes include a number of tools and endpoints to characterize the time course of asthma exacerbations. The Protocol Review Committee previously suggested that AsthmaNet trials be used to gather preliminary information on exacerbations, as was also suggested in a recent NIH Outcomes Workshop[20]. These assessments will be incorporated within the main SIENA protocol and visit structure, to minimize both participant and site burden, and to enhance safety follow-up.

5.3 Statistical Analysis

Primary Outcome

The co-primary research hypotheses are that among differential responders within the non-eosinophilic phenotype, ICS is superior to placebo and LMA is superior to placebo. In statistical terms, the null hypotheses are

(1) H_0: $p_{Eos–,ICS>Placebo} = p_{Eos–,Placebo>ICS}$

(2) H_0: $p_{Eos–,LMA>Placebo} = p_{Eos–,Placebo>LMA}$

We will apply two-sided, exact binomial tests at the 0.025 significance level (Bonferroni correction) for each of these null hypotheses. To assess potential period and seasonal effects, a sensitivity analysis will be performed by applying logistic regression models to those who had a differential response (i.e., the treatments were not equivalent), with covariates to adjust for period differences, season of enrollment, and ICS delivery (DPI/MDI).

Secondary analyses with the primary outcome include the following:

1. Comparisons within the eosinophilic phenotype in terms of the null hypotheses H_0: $p_{Eos+,ICS>Placebo} = p_{Eos+,Placebo>ICS}$ and H_0: $p_{Eos+,LMA>Placebo} = p_{Eos+,Placebo>LMA}$, which we will test via two-sided, exact binomial tests at the 0.025 significance level (Bonferroni correction) for each of these null hypotheses.
2. Comparisons between the non-eosinophilic and eosinophilic phenotypes in terms of the null hypotheses H_0: $p_{Eos–,ICS>Placebo} = p_{Eos+,ICS>Placebo}$ and H_0: $p_{Eos–,LMA>Placebo} = p_{Eos+,LMA>Placebo}$, which we will test via two-sided, 0.025 significance level Fisher exact tests (Bonferroni correction).
3. Comparison of the ICS and LMA treatments in the manner described above for the primary and secondary analyses (within the non-eosinophilic phenotype, within the eosinophilic phenotype, and between the eosinophilic and non-eosinophilic phenotypes, respectively).
4. Application of univariable and multivariable logistic regression that uses sputum eosinophils, blood eosinophils, FENO and serum periostin, as well as bronchodilator reversibility, measures of atopy, and other phenotypic characteristics from both eosinophilic and non-eosinophilic participants to construct ROC curves and c (concordance) statistics to identify “cutpoints” for each biomarker (which also can be compared with previously suggested cutpoints) to examine the value of these biomarkers as predictors of response to treatments.

All of the analyses described above will follow the intention-to-treat paradigm whereby all available data from randomized participants are included in the analyses regardless of information about deviations from study protocol.

The table below summarizes the primary and first 3 secondary analyses described above.

Hypothesis	Comparison(s)	Null Hypothesis(es)	Method	Sensitivity Analysis
Primary (EOS low)	ICS vs. PBO LAMA vs. PBO	H_0: $p_{Eos-,ICS>PBO} = p_{Eos-,PBO>ICS}$ H_0: $p_{Eos-,LMA>PBO} = p_{Eos-,PBO>LAMA}$	Exact binomial tests at 0.025 level in those with differential response for each comparison	Logistic regression for binary composite outcome defined between two treatments with adjustment for period differences and seasonal effects
Secondary 1 (EOS high)	ICS vs. PBO LAMA vs. PBO	H_0: $p_{Eos+,ICS>PBO} = p_{Eos+,PBO>ICS}$ H_0: $p_{Eos+,LMA>PBO} = p_{Eos+,PBO>LAMA}$	Exact binomial tests at 0.025 level in those with differential response for each comparison	Logistic regression for binary composite outcome defined between two treatments with adjustment for period differences and seasonal effects
Secondary 2 (EOS low vs. high)	ICS vs. PBO LAMA vs. PBO	H_0: $p_{Eos-,ICS>PBO} = p_{Eos+,ICS>PBO}$ H_0: $p_{Eos-,LMA>PBO} = p_{Eos+,LAMA>PBO}$	Fisher's exact tests at 0.025 level between those with differential response for each comparison	Logistic regression for binary composite outcome defined between two treatments with EOS group main effect and adjustment for period differences and seasonal effects
Secondary 3 (EOS low)	ICS vs. LAMA	H_0: $p_{Eos-,ICS>LAMA} = p_{Eos-,LAMA>ICS}$	Exact binomial test at 0.05 level in those with differential response	Logistic regression for binary composite outcome defined between two treatments with adjustment for period differences and seasonal effects

Secondary Outcomes

We will analyze separately each of three components of the composite outcome as secondary outcomes. We will apply a proportional hazards regression analysis for the time to treatment failure, with a random effect term (frailty) for the SIENA participant to account for the correlations within the SIENA participant [27]. The proportional hazards regression model will include fixed terms for treatment regimen, sequence, period, and season of enrollment and an additional random effect term for clinical site. We will apply a linear mixed-effects model for longitudinal data on ACDs and FEV_1, in which the longitudinal data for the model will come from week 6 (baseline), weeks 12 and 18 (first treatment period), weeks 24 and 30 (second treatment period), and weeks 36 and 42 (third treatment period). The statistical model will include

1. fixed effects for treatment regimen, sequence, period, and season of enrollment

(spring, summer, fall, winter) nested within each of the eosinophilic and non-eosinophilic phenotypes
2. a random effect for clinical site within each of the eosinophilic and non-eosinophilic phenotypes
3. a 7×7 unstructured variance-covariance matrix for the seven measurements per participant within each of the eosinophilic and non-eosinophilic phenotypes.

We will apply a similar statistical approach for the other secondary outcomes that are measured on a continuum, such as diary peak flow values and logarithmic-transformed methacholine challenge PC_{20}. We will analyze time to asthma exacerbation in a manner similar to that for time to treatment failure.

We will pursue additional secondary analyses to investigate whether baseline measurements of the biomarkers (blood eosinophils, periostin, and exhaled nitric oxide) significantly predict any of these secondary outcomes. We will achieve this by including the biomarkers in the statistical models described in the previous paragraph.

Finally, we will perform exploratory subgroup analyses of the primary and secondary outcomes within levels of gender, minority status, age group, baseline BMI, and baseline FEV_1.

6.1 Drug Supplies/Switches

All pharmaceutical companies that manufacture long-acting muscarinic antagonists and inhaled corticosteroids were invited to participate in SIENA by providing active drug and placebo for the study.

Long-Acting Muscarinic Antagonist and Placebo: Boehringer Ingelheim provided tiotropium, in the form of tiotropium Respimat, 2.5 mcg per actuation and tiotropium placebo. Participants took 2 puffs each day (total dose active drug = 5mcg). *Boehringer Ingelheim* coordinated the blinding and labeling of drug with input and assistance from the DCC.

Inhaled Corticosteroid and Placebo: Merck agreed to provide mometasone and mometasone placebo. Mometasone initially was in the form of Asmanex® DPI, 110 mcg/puff. Participants took 2 puffs twice daily (total dose active drug = 440 mcg). *Merck* coordinated the blinding of drug with information provided by the DCC. A third-party packager labelled with additional regulatory information.

Production of mometasone DPI was discontinued shortly after SIENA study start following FDA approval of mometasone MDI. Since this was a known possibility, Merck provided AsthmaNet all available active and placebo mometasone DPI devices in 2014 with the goal of providing sufficient quantities to complete the SIENA protocol. However, Merck agreed to provide additional mometasone in MDI form if that became necessary to complete SIENA. We recognized that, based on expiration dates, if recruitment was not completed by May 2016, then a switch to MDI product would be required.

The AsthmaNet Steering Committee monitored SIENA recruitment and continually reevaluate the likelihood of completing recruitment by May 2016. In November 2015 we determined that due to lagging recruitment and mometasone DPI expiration issues, a switch to mometasone MDI was necessary. *Merck* provided mometasone and mometasone placebo in the form of Asmanex® HFA, 200 mcg/puff. Participants took 1 puff twice daily (total dose active drug = 400 mcg). *Merck* coordinated the blinding of drug with information provided by the DCC. A third-party packager labelled drug with additional regulatory information.

All participants completed the study using whichever formulation they received at randomization. No participants switched from DPI to MDI during the course of the study. The randomization plan and the statistical analysis plan were modified accordingly. In particular, we inserted an additional level of stratification for randomization according to DPI/MDI assignment.

Contingent Statistical Analysis

Because SIENA invokes a three-way crossover design, a stratified randomization based on prognostic factors is not critical. Instead, we only invoked clinical site within phenotype (eosinophilic, non-eosinophilic) as a stratifying variable with permuted blocks of size six (one complete cycle of the six). As indicated above, we included a DPI/MDI switch as another stratification variable. In particular, the stratification was according to DPI/MDI status nested within clinical center, which is nested within phenotype.

The statistical analysis plan for the primary and secondary outcomes is described in the Methods section of the Protocol. We accounted for the possible effects of DPI/MDI status by including it as another covariate in the sensitivity analysis for the composite outcome.

We do not believe that the switch from active DPI to active MDI negatively impacted the scientific validity of the study.

6.2 Missing Data Analysis

Comparison	Result	Observed	Assume missing = tied	Assume missing at random	Tipping point: assume missing favors ICS 64/36 (%)
ICS vs. PBO	ICS>PBO	74	74	93	103
	PBO>ICS	56	56	70	72
	TIED	46	91	58	46
	MISSING	45			
	P-value		0.136	0.085	0.023
		Observed	**Assume missing = tied**	**Assume missing at random**	
LAMA vs. PBO	LAMA>PBO	79	79	97	
	PBO>LAMA	53	53	65	
	TIED	49	89	60	
	MISSING	40			
	P-value		0.029	0.015	

The column labeled 'Assume missing = tied' illustrates the ITT analysis results reported in the manuscript. The next column shows what would be expected under the missing at random assumption where the imputed responses reflect the percentages observed in the data, and statistical significance is achieved for the LAMA vs. PBO comparison under this assumption. The last column reflects a tipping point analysis in which we show what percentage of the missing differential responses favoring the active treatment would have been needed to switch our results from non-significant to significant for the ICS comparison. For the ICS vs Placebo comparison, 64% of the missing differential responses favoring ICS would have been needed to achieve a p-value less than 0.025, which is markedly higher than we observed in the non-missing data (57%). We do not show a tipping point for the LAMA comparison as that is significant under the missing at random assumption.

Of the 54 drop-outs (Figure 2, EOS LOW and EOS HIGH), 20 dropped out in the first period and are missing for all 3 comparisons. Of the remaining 34 drop-outs, 32 were missing from the ICS vs. PBO comparison, and 29 were missing from the LAMA vs. PBO comparison. The table below shows summary statistics for each component of the outcome for those subjects who were not missing from either comparison, along with any available outcome data on those participants not included in either the ICS vs PBO or the LAMA vs PBO comparisons. Focusing on the overall estimates for the placebo period, we are reassured at the similarity of the results for those who were not missing from either comparison (column 3), and those who were not included in either treatment vs PBO comparison (column 4).

Descriptive statistics of outcome data to address missing at random assumption: (EOS LOW and EOS HIGH)

		Those not missing from either comparison (N=241)	Those missing from either treatment vs PBO comparison (N=13)
Components of outcome	**Treatment**	**N (%) or mean (SD)**	**N (%) or mean (SD)**
Treatment Failure	PBO	27 (11.2%)	22 (15.4%)
Annualized ACD	PBO	180.5 (137.4)	161.5 (130.8)
FEV_1 (% of predicted)	PBO	91.8 (13.6)	88.2 (16.4)

7.1 Adherence

Adherence to diary recording and to scheduled medications was monitored using the Spirotel® electronic diary, and counters built into the inhaler devices. Alerts built into the Spirotel® prompted participants to take their medications and record their symptoms.

Spirotel® Device

The Spirotel® device is an electronic diary (e-diary) and peak flow monitor in one unit that stores in its memory all measurements the participant provided between visits. The device was customized for AsthmaNet to provide a participant-friendly screen and flow of procedures. Participants had defined windows during which they could do their morning and evening assessments, including answering their diary questions and performing their peak flow maneuvers. This device does not allow 'backfilling' or 'recall' of data; it must be used on schedule twice daily. This customization required participants to be conscientious about their home activities in order to meet the compliance thresholds required for the study.

Data from the Spirotel® device were downloaded at each visit and reports were generated for review with the participant. The Spirotel® Participant Visit Report shows the dates and times associated with each AM and PM session, along with the diary data the participant entered and his/her PEF measurements. The Spirotel® Participant Compliance Report (P6_COMPLY_RPT) provided metrics on how frequently the participant carried out all required home procedures between visits. Knowing that e-diary data would be reviewed at the next visit helped to encourage participants to be more compliant. Daily diary records help participants assume more responsibility for their own care. Recall bias is minimized, as the e-diary device requires participants to complete their AM and PM diary assessments each day.

Specific SIENA 'alerts' were programmed into the Spirotel® device. These alerts prompted participants to take their morning and evening medications, start open-label Asmanex® (YELLOW) inhaler for treatment failure, and call the clinic. These alerts were intended to improve adherence with several aspects of the protocol.

Peak flow measurement and diary question completion were important daily activities. Regular measurement of lung function and assessment of symptoms and rescue inhaler use were intended to help the participant identify when he/she was trending towards exacerbation and increase adherence with the onset of appropriate treatment and reporting of these events.

Improper peak flow technique is a form of non-adherence. Coaching the participant on the proper technique early in the study and reviewing this technique throughout the study improve adherence. The Spirotel® Performance Checklist (SPIROTEL_PERF) was used at Visit 1 (or Visit 0A for Supervised Washout participants) to document that each participant had achieved proper peak flow technique.

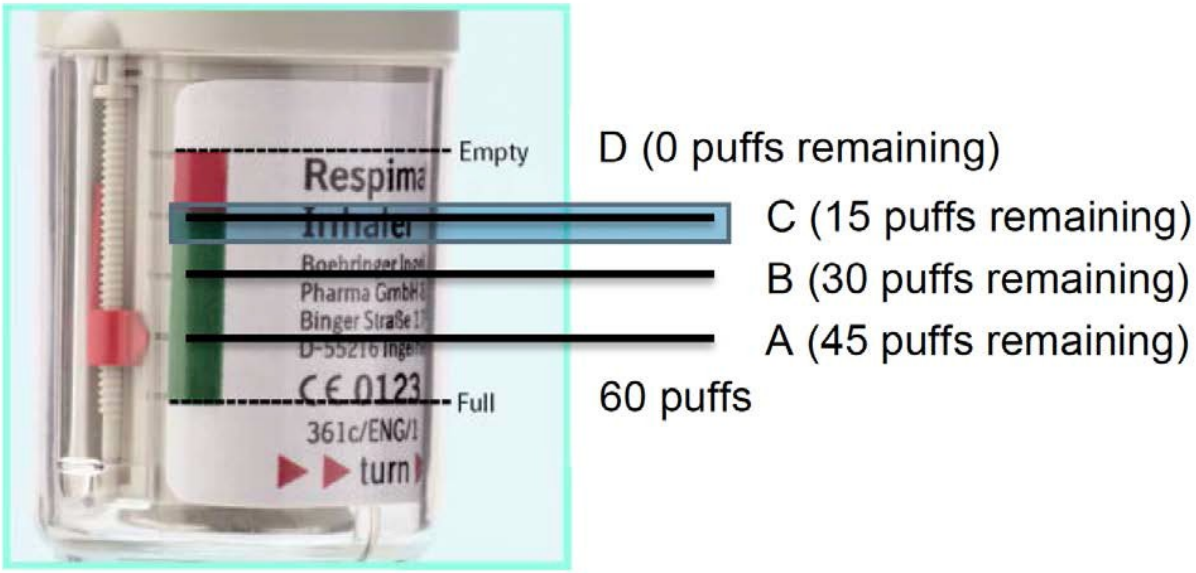
Failure to complete diary assessments twice a day is another form of non-adherence. Instructing the participant in the proper way to use the spirotel® device for entry of diary information improves adherence. The SPIROTEL_PERF checklist was used at Visit 1 (or Visit 0A for Supervised Washout participants) to document that each participant had achieved an understanding of how to use the spirotel® device correctly.

At each visit the participant's level of adherence with study procedures was assessed. Individuals who maintained high levels of adherence were congratulated. If adherence levels were low, this was addressed with the participant.

Dosing Compliance

Tiotropium: The Respimat® has an indicator that shows the number of puffs remaining (out of a total of 60 puffs in a new Respimat®). This indicator was used to assess the participant's compliance with dosing from the Respimat® during the run-in and randomized treatment phase. For both the run-in and randomized treatment phase, participants were instructed to take 2 puffs once daily in the morning from the Respimat®.

The indicator on the Respimat® appears as follows:



After the Respimat® is programmed for the first-time, the device will have 60 puffs (30 doses). The red indicator will be at the bottom of the indicator mark (labeled "Full" in the picture, with 60 puffs remaining.

Since there is no dose counter on the device, the markings were used to calculate compliance, noting that the indicator line marked "A" represents 45 puffs remaining, the indicator line marked "B" represents 30 puffs remaining, the indicator line marked "C" represents 15 puffs remaining, and the indicator line marked "D" or "Empty" represents 0 puffs remaining. To best estimate the number of puffs remaining on the device the research coordinators "dumped" puffs until one of these markings was reached. The number of puffs remaining in the device was then equal to the number of "dumped" puffs + the number of puffs remaining based on the indicator. If puffs were "dumped" to get to line:

- A: # of remaining puffs = # of "dumped" puffs + 45
- B: # of remaining puffs = # of "dumped" puffs + 30
- C: # of remaining puffs = # of "dumped" puffs + 15
- D: # of remaining puffs = # of "dumped" puffs + 0

The number of scheduled puffs included all doses the participant should have taken since leaving the last clinic visit.

Mometasone: The Twisthaler® device contains a counter that shows the number of puffs remaining (out of a total of 60 puffs in a new Twisthaler®). This counter was used to assess the participant's compliance with dosing from the Twisthaler® during the randomized treatment phase.

The mometasone MDI device contains a counter that shows the number of puffs remaining (out of a total of 120 puffs in a new MDI). This counter was used to assess the participant's compliance with dosing from the MDI during the randomized treatment phase.

The time-stamps on the Spirotel® for diary entries and medication use served as a check on the counters attached to the inhalers.

Observed Adherence to medications by stratum

	ICS	LAMA	PBO
EOS Low	84.6%	83.4%	85.2%
EOS High	85.4%	84.7%	86.2%

Observed Adherence to diary completion by stratum

	ICS	LAMA	PBO
EOS Low	64.2%	65.9%	63.5%
EOS High	69.4%	70.2%	67.1%

8.1 Figures

Figure S1. Eosinophil High Stratum

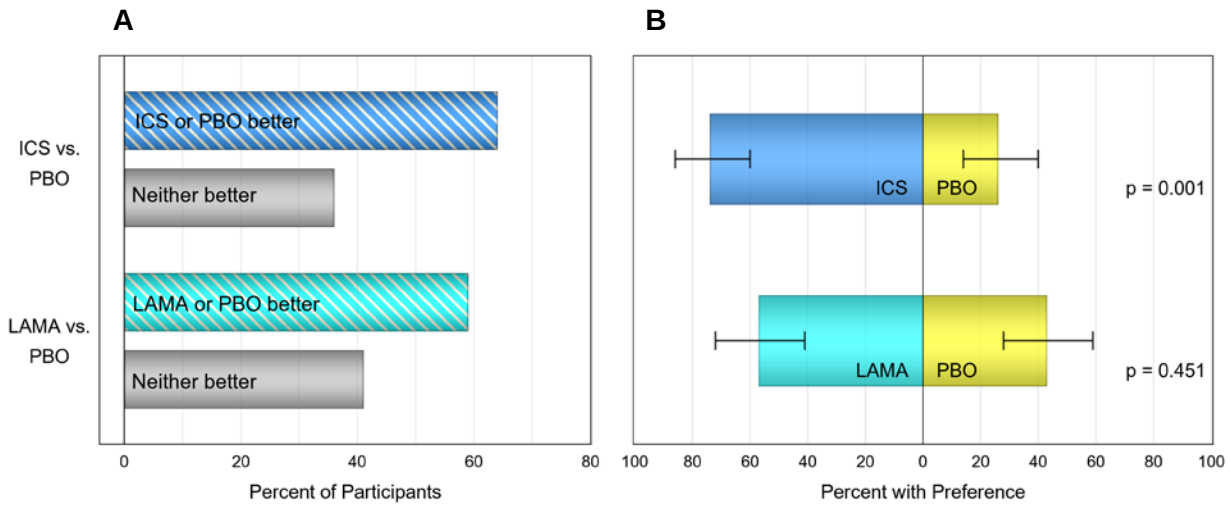


Figure S1: Pairwise comparison of active treatments and placebo, using composite of hierarchical outcomes (Treatment Failure, Annualized Asthma Control Days, FEV1).

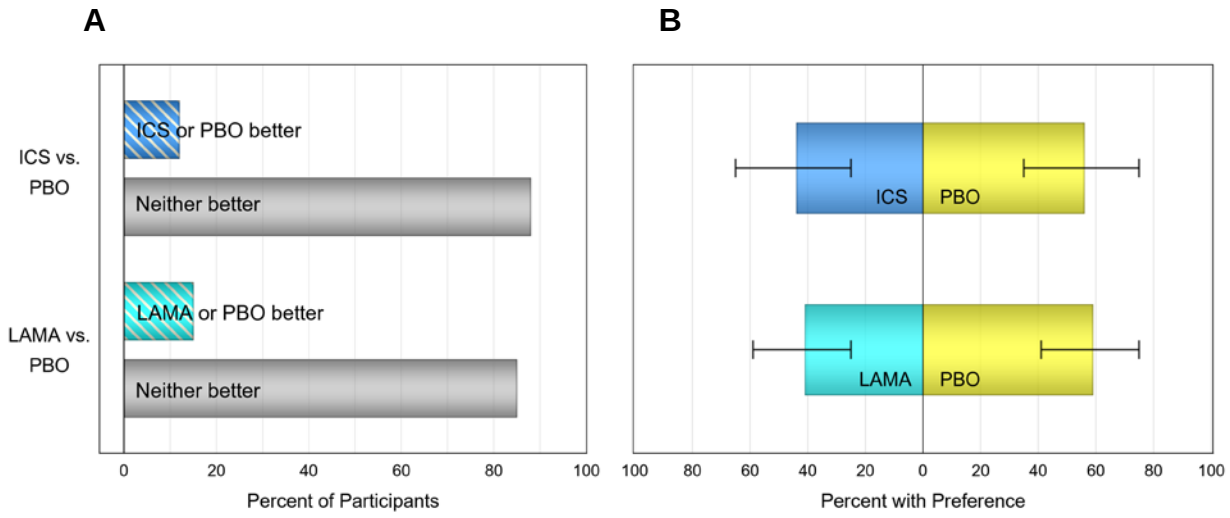
Panel A shows the proportion of participants with ≥2% sputum eosinophils who did better on the hierarchical composite when treated with ICS vs PBO or LAMA vs PBO. A participant was considered to have had a differential response if at least one treatment period was ranked better than another. Either a more favorable response to an active treatment vs PBO or to PBO vs an active treatment was counted as a differential reponse.

Panel B shows significantly more Eos High participants with a differential response had a better reponse to ICS (74%) than to PBO (26%), whereas the responses to LAMA and PBO were not significantly different (57% vs 43%).

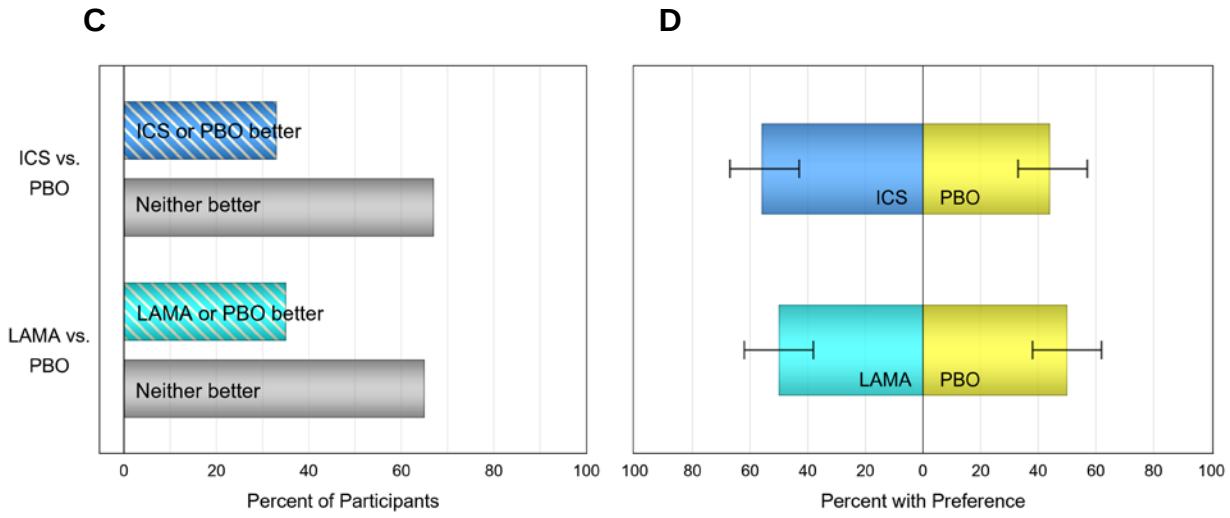
Bars represent 95% Confidence Intervals.

Figure S2 – Eos Low

Treatment Failure



AACD



FEV1

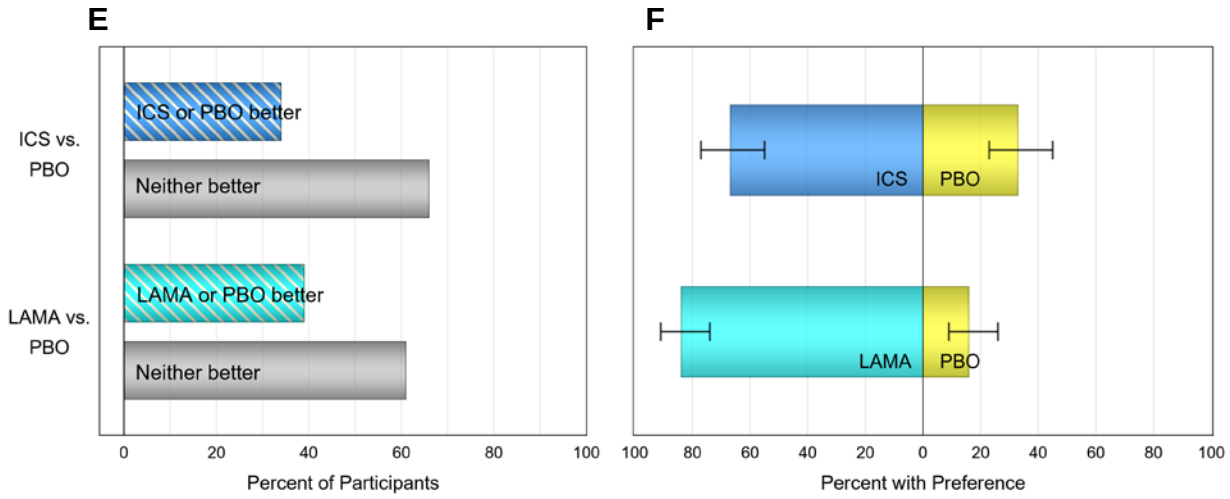


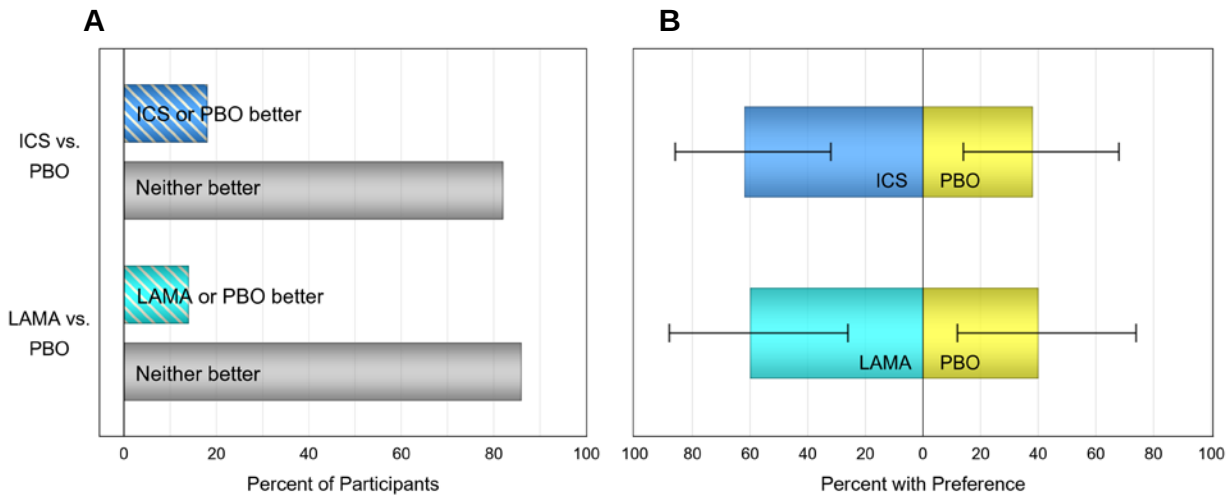
Figure S2: Pairwise comparison of active treatments and placebo, showing individual components of the hierarchical outcomes (See Manuscript Figure 2).

Panel A shows the proportion of participants with <2% sputum eosinophils who had fewer Treatment Failures when treated with ICS vs PBO or LAMA vs PBO. **Panel B** shows the statistical comparison between participants with a differential response. The percentage with a better response to ICS or PBO was not significantly different (p=0.70), nor was the percentage who had a better response to LAMA and PBO (p=0.39). **Panel C** shows the percentage of Eos Low participants who had more Annualized ACDs when treated with ICS vs PBO or LAMA vs PBO. **Panel D** shows no significant difference in AACDs between ICS and PBO (p=0.41) or LAMA and PBO (p=1.00). **Panel E** shows the percentage whose FEV1 improved better on ICS vs PBO or LAMA vs PBO. **Panel F** shows that significantly more participants had a better response to ICS vs PBO, and LAMA vs PBO.

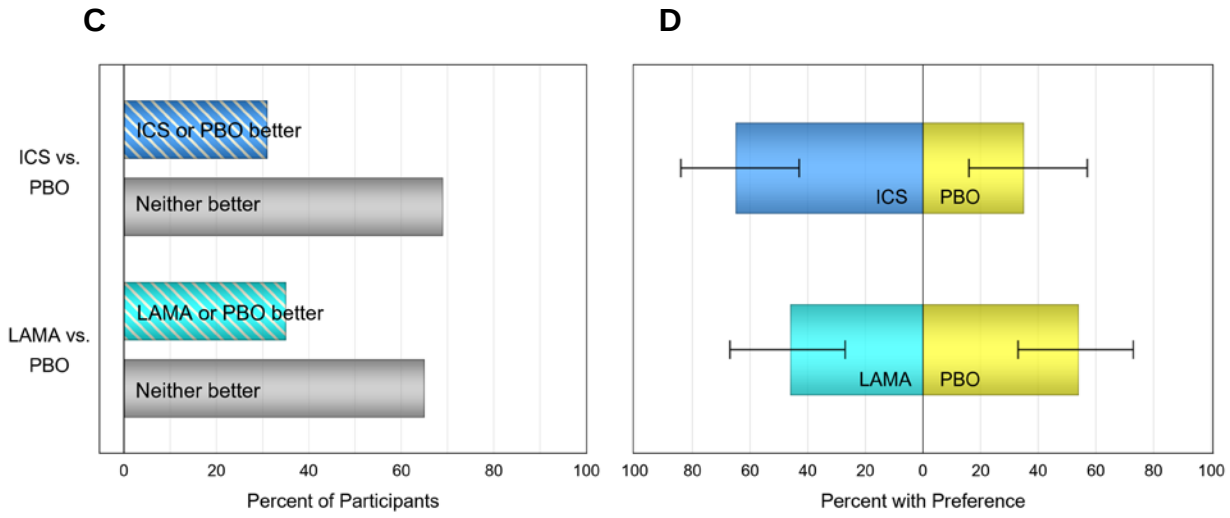
Bars represent 95% Confidence Intervals.

Figure S3 – Eos High

Treatment Failure



AACD



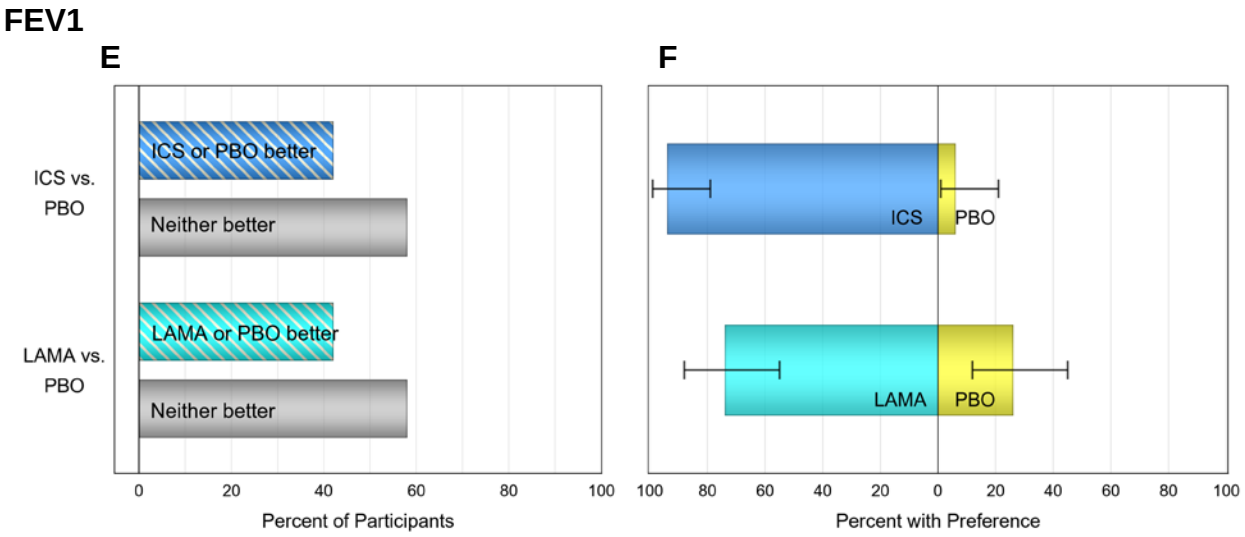


Figure S3: Pairwise comparison of active treatments and placebo, showing individual components of the hierarchical outcomes (See Manuscript Figure 3). **Panel A** shows the percentage of participants with ≥2% sputum eosinophils who had fewer Treatment Failures when treated with ICS vs PBO or LAMA vs PBO. **Panel B** shows the statistical comparison between participants with a differential response. The percentage with a better Treatment Failure response to ICS was not significantly greater than those who responded to PBO, nor was the percentage who had a better response to LAMA and PBO. **Panel C** shows the percentage of Eos High participants who had more Annualized ACDs when treated with ICS vs PBO or LAMA vs PBO. **Panel D** shows no significant difference in AACDs between ICS and PBO or LAMA and PBO. **Panel E** shows the percentage whose FEV1 improved better on ICS vs PBO or LAMA vs PBO. **Panel F** shows that significantly more participants had a better FEV1 response to ICS vs PBO, and LAMA vs PBO. Bars represent 95% Confidence Intervals.

Figure S4.

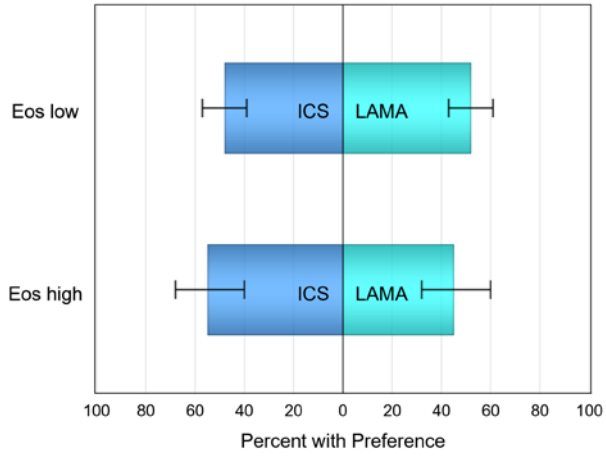


Figure S4: Pairwise comparison of ICS vs LAMA in the Eos low and Eos high strata, using composite of hierarchical outcomes (Treatment Failure, Annualized Asthma Control Days, FEV_1).

There was no significant difference in the response to ICS vs LAMA in either the Eos low (48% vs 52%) or Eos high (55% vs 45%) strata. Bars represent 95% Confidence Intervals.

Figure S5.

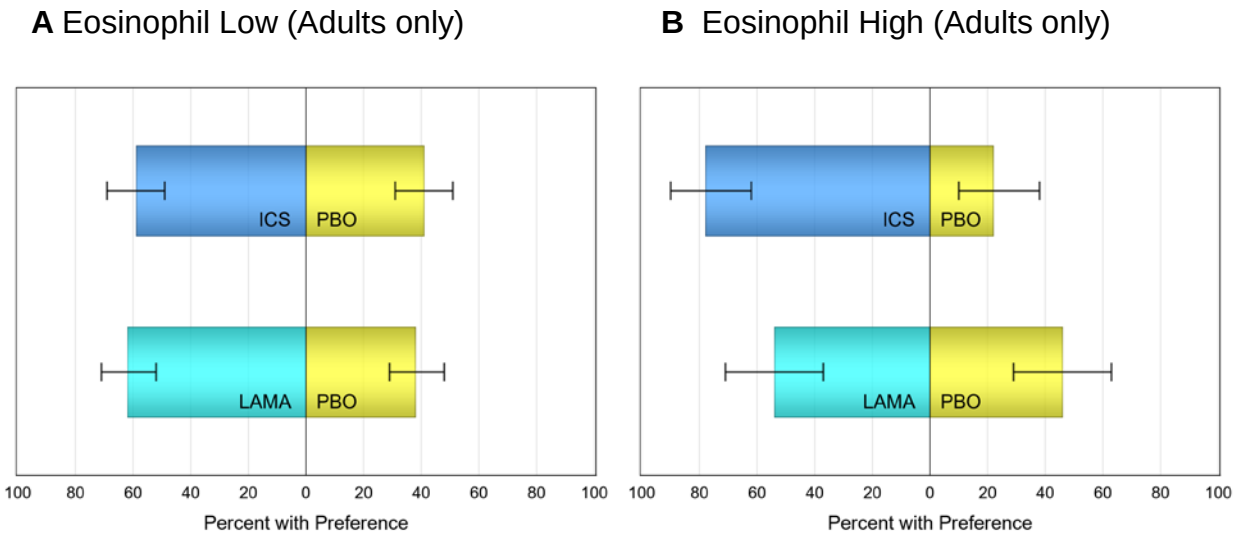


Figure S5: Pairwise comparison of active treatments and placebo in adults.

Panel A: In adults with <2% sputum eosinophils and a differential response, there was no significant difference between the response to ICS vs PBO (57% vs. 43%), but a significantly greater proportion reponded better to LAMA vs PBO (60% vs. 40%). **Panel B**: In adults with ≥2% sputum eosinophils and a differential response, the proportion who responded better to ICS was significantly greater than that responding to PBO (74% vs. 26%) but there was no difference for the response to LAMA vs PBO (57% vs. 43%). Bars represent 95% Confidence Intervals.

Figure S6

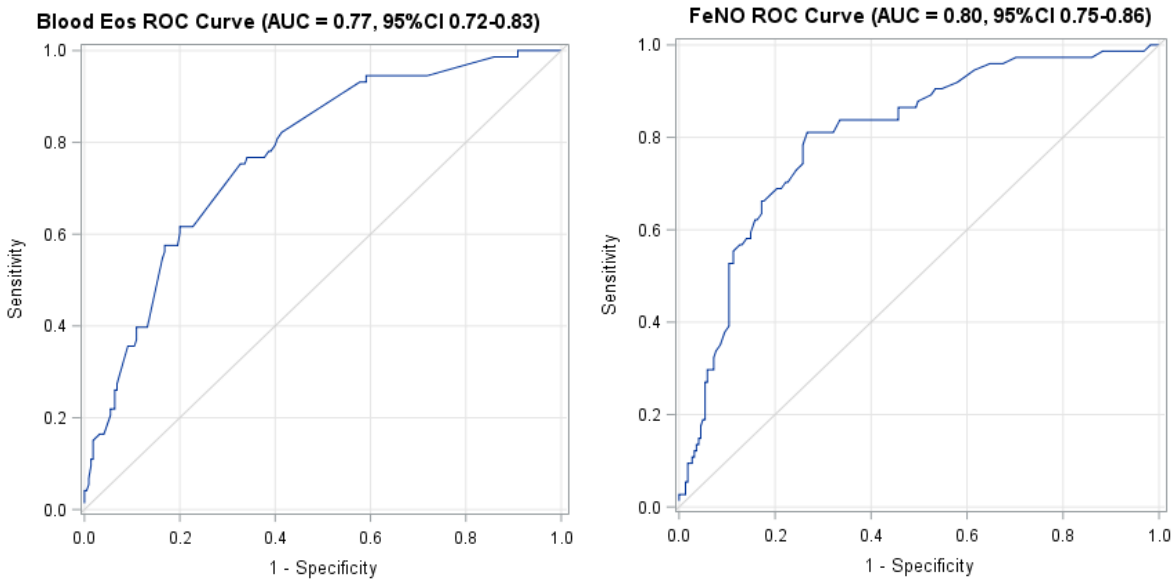


Figure S6: Receiver operating characteristic curve analysis of the sensitivity and specificity of blood eosinophils and Fe_{NO} for sputum eosinophil status.

Figure S7

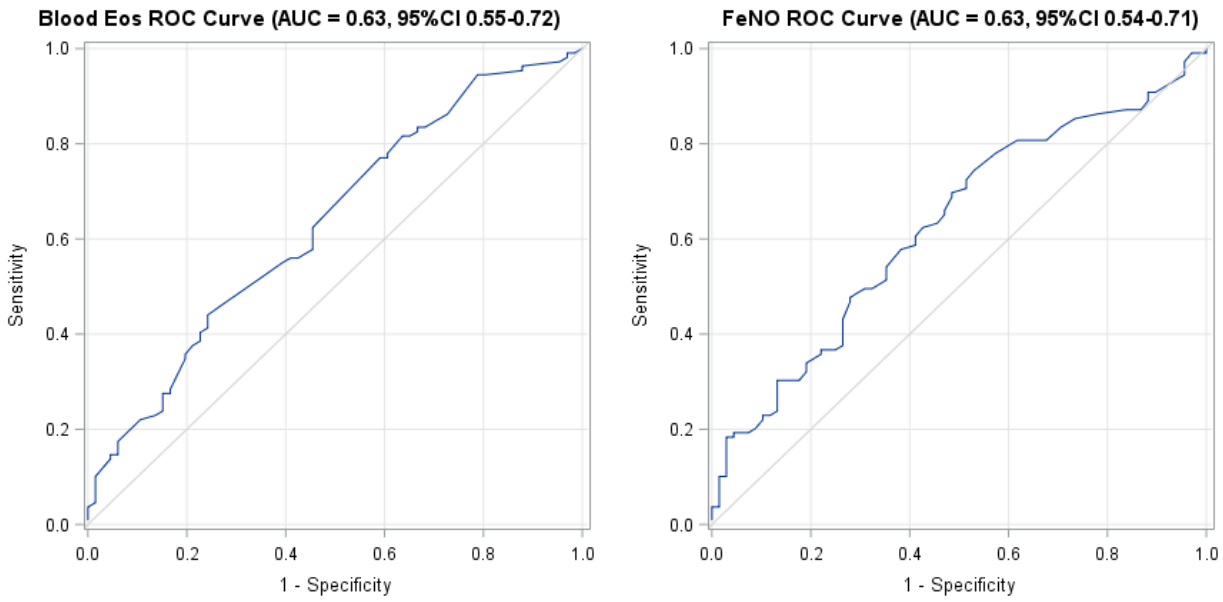


Figure S7: Receiver operating characteristic curve analysis of the sensitivity and specificity of blood eosinophils and FeNO for response to ICS therapy.

Figure S8

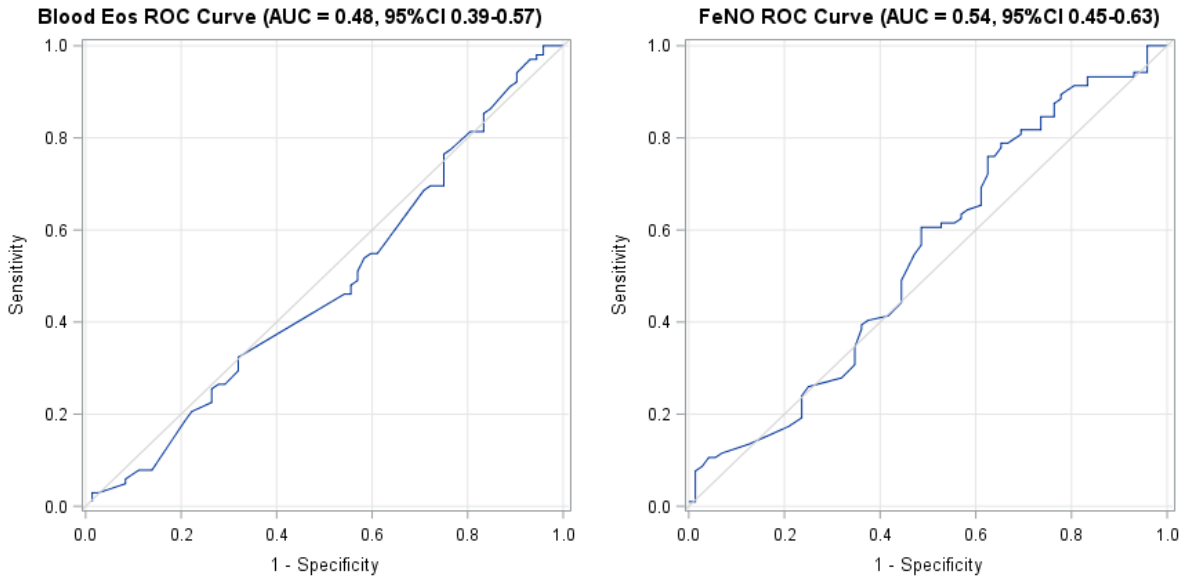


Figure S8: Receiver operating characteristic curve analysis of the sensitivity and specificity of blood eosinophils and Fe_{NO} for response to LAMA therapy.

Figure S9

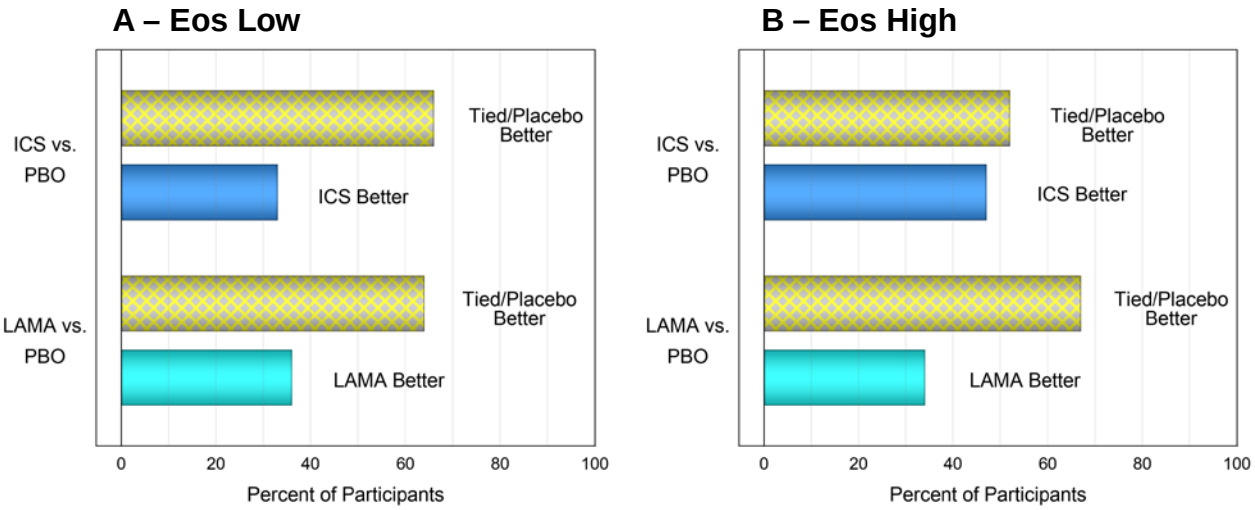


Figure S9A: Comparison of Active Treatments and Placebo in Eosinophil Low Stratum
When participants who responded better to PBO (25%) and those who showed no difference between ICS and PBO ("tied", 41%) were combined (66%), the comparison with those who responded better to ICS (33%) was non-significant. For the LAMA vs PBO comparison, 24% responded better to PBO, 40% showed no difference between LAMA and PBO ("tied") and the combination of tied and PBO better (64%) was not significantly different than LAMA better (36%).

Figure S9B: Comparison of Active Treatments and Placebo in Eosinophil High Stratum
When participants who responded better to PBO and those who showed no difference between ICS and PBO ("tied") were combined, the comparison with those who responded better to ICS was not significant. For the LAMA vs PBO comparison, the combination of those who showed no difference between LAMA and PBO ("tied") and those who responded better to PBO was not significantly different than LAMA better.

9.1 Tables

Table S1. Baseline Characteristics of SIENA Randomized Participants

Characteristic[†]	Eosinophil Low (N=221)	Eosinophil High (N=74)
Demographics		
Age at enrollment	31.2±13.8	31.1±14.2
Male – no. of participants (%)	76 (34.4%)	35 (47.3%)
Race/Ethnicity – no. of participants (%):		
Asian/PI	7 (3.2%)	4 (5.4%)
Black	71 (32.1%)	17 (23.0%)
White	130 (58.8%)	38 (51.4%)
Hispanic	12 (5.4%)	12 (16.2%)
Other	1 (0.5%)	3 (4.1%)
Asthma History		
Median Age when doctor first diagnosed (interquartile range)	8.0 (3.0-15.0)	7.0 (3.0-14.0)
Duration of asthma (years since doctor first diagnosed)	19.2±10.9	20.0±12.2
Family History of Asthma – no. of participants (%)	142 (68.3%)	51 (68.9%)
Prior Year – no. of participants (%):		
One or more asthma episodes requiring emergency care or unscheduled office visit	52 (23.6%)	17 (23.0%)
One or more overnight hospitalizations due to asthma	4 (1.8%)	3 (4.1%)
One or more courses of systemic corticosteroid therapy taken for asthma	41 (18.6%)	14 (18.9%)
Days of work, school, or housework missed due to asthma:		
0 days	141 (64.4%)	48 (64.9%)
1 to 7 days	58 (26.5%)	21 (28.4%)
> 7 days	20 (9.1%)	5 (6.8%)
ICS (not including combination meds)	45 (20.5%)	16 (21.6%)
ICS/LABA Combination Therapy	25 (11.4%)	4 (5.4%)
Inhaled Muscarinic Antagonist	3 (1.4%)	2 (2.7%)
LTRA / 5LO Inhibitors	24 (10.9%)	7 (9.5%)
Clinical and spirometric features		
BMI at enrollment (kg/m^2)	29.1±7.8	26.5±5.7
FEV_1 % predicted at randomization	92.7±12.4	89.5±10.8
FEV_1/FVC ratio at randomization	0.77±0.08	0.75±0.08
PC_{20} (mg/ml) at enrollment – geometric mean ± CV	2.42±1.28	1.24±1.27
Bronchodilator Response (4 puffs) at enrollment (relative % change)	9.6±7.1	12.7±8.5
Median eNO (ppb) at enrollment (interquartile range)	21.5 (14.0-35.5)	55.5 (35.0-81.0)

Characteristic†	Eosinophil Low (N=221)	Eosinophil High (N=74)
Median Blood Eosinophils (%) at enrollment (interquartile range)	2.6 (1.1-4.0)	4.8 (3.9-7.0)
Median Periostin (ng/mL) at enrollment (interquartile range)	51.7 (43.3-63.6)	56.3 (49.3-75.2)
Median ACT Score at randomization[1] (interquartile range)	21.0 (20.0-23.0)	21.0 (19.0-23.0)
Eczema/atopic dermatitis (physician-diagnosed) – no. of participants (%)	67 (30.4%)	27 (36.5%)
≥1 Positive allergen test – no./total no. (%)	172/216 (79.6%)	70/72 (97.2%)
Number of positive allergen tests		
0	44 (20.4%)	2 (2.8%)
1	10 (4.6%)	1 (1.4%)
2	26 (12.0%)	3 (4.2%)
3+	136 (63.0%)	66 (91.7%)

† Means ± SD presented unless otherwise noted.
1 Individual ACT questions are scaled 1 to 5, with higher values representing better asthma control. ACT score is sum of questions 1-5.

Table S2. Baseline Characteristics of SIENA Randomized *Adolescent* Participants

	Eosinophil Low (N=40)	Eosinophil High (N=18)
Characteristic[†]	**N (%)**	**N (%)**
Demographics		
Age at enrollment	14.5±1.6	15.1±1.4
Male – no. of participants (%)	21 (52.5%)	10 (55.6%)
Race/Ethnicity – no. of participants (%):		
Black	21 (52.5%)	6 (33.3%)
White	15 (37.5%)	6 (33.3%)
Hispanic	3 (7.5%)	5 (27.8%)
Other	1 (2.5%)	1 (5.6%)
Asthma History		
Median Age when doctor first diagnosed (interquartile range)	3.0 (2.0-6.0)	3.5 (2.0-7.0)
Duration of asthma (years since doctor first diagnosed)	10.5±3.7	10.5±3.8
Family History of Asthma – no. of participants (%)	28 (73.7%)	15 (83.3%)
Prior Year – no. of participants (%):		
One or more asthma episodes requiring emergency care or unscheduled office visit	15 (37.5%)	8 (44.4%)
One or more overnight hospitalizations due to asthma	2 (5.0%)	1 (5.6%)
One or more courses of systemic corticosteroid therapy taken for asthma	12 (30.0%)	8 (44.4%)
Days of work, school, or housework missed due to asthma:		
0 days	17 (42.5%)	9 (50.0%)
1 to 7 days	13 (32.5%)	8 (44.4%)
> 7 days	10 (25.0%)	1 (5.6%)
ICS (not including combination meds)	11 (27.5%)	8 (44.4%)
ICS/LABA Combination Therapy	5 (12.5%)	1 (5.6%)
Inhaled Muscarinic Antagonist	1 (2.5%)	2 (11.1%)
LTRA / 5LO Inhibitors	9 (22.5%)	2 (11.1%)
Clinical and spirometric features		
BMI at enrollment (kg/m^2)	25.2±7.4	23.6±4.3
FEV_1 % predicted at randomization	96.2±11.9	92.1±10.9
FEV_1/FVC ratio at randomization	0.80±0.07	0.78±0.08
PC_{20} (mg/ml) at enrollment – geometric mean ± CV	2.67±1.41	1.06±1.04
Bronchodilator Response (4 puffs) at enrollment (relative % change)	11.3±7.2	11.5±5.4

	Eosinophil Low (N=40)	Eosinophil High (N=18)
Characteristic†	**N (%)**	**N (%)**
Median eNO (ppb) at enrollment (interquartile range)	25.0 (18.0-37.0)	65.5 (43.0-103.0)
Median Blood Eosinophils (%) at enrollment (interquartile range)	3.0 (1.0-4.1)	6.2 (3.3-7.9)
Median Periostin (ng/mL) at enrollment (interquartile range)	89.1 (68.7-124.7)	98.1 (65.9-106.4)
Median ACT Score at randomization[1] (interquartile range)	23.0 (21.0-24.0)	20.5 (19.0-22.0)

†Means ± SD presented unless otherwise noted.
1 Individual ACT questions are scaled 1 to 5, with higher values representing better asthma control. ACT score is sum of questions 1-5.

Table S3. Questionnaires/Diary Data

Questionnaire*	ICS	LAMA	Placebo
Eos Low			
Asthma Bother Profile (ABP)	16.9 (13.4, 20.3)	16.6 (13.1, 20.0)	16.6 (13.2, 20.1)
Asthma Control Test (ACT)	21.8 (21.4, 22.2)	21.6 (21.3, 22.0)	21.7 (21.4, 22.1)
Asthma Symptom Utility Index (ASUI)	0.85 (0.81, 0.90)	0.86 (0.81, 0.91)	0.86 (0.81, 0.91)
Sinonasal Questionnaire (SNQ)	1.00 (0.81, 1.19)	0.97 (0.78, 1.16)	0.96 (0.77, 1.16)
Asthma-Specific Work Productivity and Activities Impairment Questionnaire (WPAI: Asthma)	7.4 (5.1, 9.6)	8.4 (6.2, 10.6)	6.8 (4.6, 9.0)
DIARY: morning peak expiratory flow	437.2 (431.8, 442.6)	444.3 (438.9, 449.7)	432.6 (427.2, 438.0)
DIARY: evening peak expiratory flow	441.3 (436.0, 446.5)	453.0 (447.7, 458.2)	437.6 (432.3, 442.9)
DIARY: nocturnal awakenings	0.013 (0.008, 0.019)	0.013 (0.008, 0.018)	0.009 (0.005, 0.014)
Eos High			
Asthma Bother Profile	15.1 (11.4, 18.9)	15.0 (11.2, 18.8)	16.5 (12.8, 20.2)
Asthma Control Test	22.3 (21.7, 22.9)	21.4 (20.8, 22.0)	21.6 (21.0, 22.1)
Asthma Symptom Utility Index	0.89 (0.84, 0.94)	0.84 (0.79, 0.90)	0.84 (0.79, 0.89)
Sinonasal Questionnaire	0.96 (0.75, 1.18)	1.03 (0.81, 1.25)	1.04 (0.82, 1.25)
Asthma-Specific Work Productivity and Activities Impairment Questionnaire (WPAI: Asthma)	5.7 (2.1, 9.4)	6.7 (2.8, 10.5)	5.5 (1.8, 9.3)
DIARY: morning peak expiratory flow	446.1 (437.4, 454.8)	444.5 (435.7, 453.3)	431.8 (423.1, 440.6)
DIARY: evening peak expiratory flow	445.3 (436.8, 453.9)	451.9 (443.3, 460.6)	437.7 (429.2, 446.3)
DIARY: nocturnal awakenings	0.005 (0.001, 0.011)	0.005 (0.001, 0.012)	0.007 (0.002, 0.014)

*ABP: The scores on the ABP range from 0 to 75 and higher scores indicate poorer quality of life; ACT: The scores on the ACT range from 5 (uncontrolled) to 25 (well-controlled), higher scores indicate better asthma control and the minimal clinically important difference is 3; ASUI: The scores on the ASUI range from 0 to 1; a higher score indicates better symptom control (0.88, mild; 0.64, moderate; and 0.47, severe asthma) and the minimal clinically important difference is 0.09; SNQ: The scores on the SNQ range from 0 (never) to 3 (daily), and a score of 1 or greater is highly sensitive and specific in determining presence of sinonasal disease; WPAI: WPAI outcomes are expressed as impairment percentages, with higher numbers indicating greater impairment and less productivity, i.e., worse outcomes.

Table S4. Adverse Events for All Participants (Eos Low and Eos High)

	ICS (n=255)	LAMA (n=258)	Placebo (n=254)
Complications Of Pregnancy, Childbirth, And The Puerperium			
Spontaneous Abortion	1	0	0
Diseases Of The Digestive System			
Constipation	0	1	0
Diseases Of The Nervous System And Sense Organs			
Migraine	0	1	0
Diseases Of The Respiratory System			
Acute nasopharyngitis	4	0	0
Acute pharyngitis	1	0	0
Acute upper respiratory infection	0	0	1
Diseases Of The Skin And Subcutaneous Tissue			
Contact dermatitis and other eczema	0	1	0
Infectious And Parasitic Diseases			
Candidiasis	2	1	0
Symptoms, Signs, And Ill-Defined Conditions			
Epistaxis	0	1	0
Rash and other nonspecific skin eruption	0	1	0
Respiratory abnormality, unspecified	1	1	0
Swelling, mass, or lump in head and neck	0	1	0

Table S5. Treatment Failures and Asthma Exacerbations for All Participants (Eos Low and Eos High)

	ICS (n=255)	LAMA (n=258)	Placebo (n=254)
Treatment Failure			
0	226 (89%)	223 (86%)	225 (89%)
1	24 (9%)	29 (11%)	22 (9%)
2	5 (2%)	6 (2%)	7 (3%)
Exacerbations leading to systemic steroids			
0	252 (99%)	253 (98%)	253 (99.6%)
1	3 (1%)	5 (2%)	1 (0.4%)

Table S6: Baseline data for the assessment of those with acceptable vs. unacceptable sputum samples in the run-in

Characteristic[†]	Eos Group Undetermined (N=198)	Eos Group Determined (N=366)
Demographics		
Age at enrollment	28.5±13.6	30.7±13.5
Male – no. of participants (%)	60 (30.3%)	137 (37.4%)
Race/Ethnicity – no. of participants (%):		
AI/AN	0 (0.0%)	2 (0.5%)
Asian/PI	5 (2.5%)	13 (3.6%)
Black	78 (39.4%)	120 (32.8%)
White	85 (42.9%)	193 (52.7%)
Hispanic	28 (14.1%)	31 (8.5%)
Other	2 (1.0%)	7 (1.9%)
Asthma History		
Median Age when doctor first diagnosed (interquartile range)	6.0 (2.0-15.0)	8.0 (3.0-14.0)
Duration of asthma (years since doctor first diagnosed)	17.5±11.2	19.1±10.6
Family History of Asthma – no. of participants (%)	136 (70.5%)	244 (69.9%)
Prior Year – no. of participants (%):		
One or more asthma episodes requiring emergency care or unscheduled office visit	56 (28.4%)	94 (25.8%)
One or more overnight hospitalizations due to asthma	3 (1.5%)	9 (2.5%)
One or more courses of systemic corticosteroid therapy taken for asthma	42 (21.3%)	68 (18.6%)
Days of work, school, or housework missed due to asthma:		
0 days	123 (62.4%)	228 (62.6%)
1 to 7 days	55 (27.9%)	98 (26.9%)
> 7 days	19 (9.6%)	38 (10.4%)
ICS (not including combination meds)	48 (24.4%)	75 (20.7%)
ICS/LABA Combination Therapy	21 (10.7%)	36 (9.9%)
Inhaled Muscarinic Antagonist	4 (2.0%)	5 (1.4%)
LTRA / 5LO Inhibitors	32 (16.2%)	33 (9.0%)
Clinical and spirometric features		
BMI at enrollment (kg/m^2)	27.3±8.2	28.9±7.8
FEV_1 % predicted at randomization		91.4±12.6

	Eos Group Undetermined (N=198)	Eos Group Determined (N=366)
Clinical and spirometric features		
FEV_1/FVC ratio at randomization		0.76±0.09
PC_{20} (mg/ml) at enrollment – geometric mean ± CV	1.62 (1.46)	2.02 (1.31)
Bronchodilator Response (4 puffs) at enrollment (relative % change)	10.3±8.3	10.9±7.9
Median eNO (ppb) at enrollment (interquartile range)	26.5 (15.0-58.0)	26.0 (15.0-53.0)
Median Blood Eosinophils (%) at enrollment (interquartile range)	3.0 (1.8-5.8)	3.0 (1.8-5.0)
Median Periostin (ng/mL) at enrollment (interquartile range)	57.7 (46.9-74.3)	53.0 (43.9-64.6)
Median ACT Score at randomization[1] (interquartile range)		21.0 (19.0-23.0)

† Means ± SD presented unless otherwise noted.
1 Individual ACT questions are scaled 1 to 5, with higher values representing better asthma control. ACT score is sum of questions 1-5.

Table S7: Assessment of acceptable vs. unacceptable sputum samples in the run-in by site

Site	Eos Group Undetermined (N=198)	Eos Group Determined (N=366)
111	19 (30.2%)	44 (69.8%)
112	0 (0.0%)	2 (100.0%)
113	10 (50.0%)	10 (50.0%)
121	5 (38.5%)	8 (61.5%)
122	0 (0.0%)	3 (100.0%)
123	4 (66.7%)	2 (33.3%)
125	8 (33.3%)	16 (66.7%)
126	12 (37.5%)	20 (62.5%)
131	6 (16.7%)	30 (83.3%)
132	5 (38.5%)	8 (61.5%)
142	20 (22.7%)	68 (77.3%)
151	5 (26.3%)	14 (73.7%)
153	2 (50.0%)	2 (50.0%)
154	5 (29.4%)	12 (70.6%)
161	30 (51.7%)	28 (48.3%)
171	8 (24.2%)	25 (75.8%)
172	2 (66.7%)	1 (33.3%)
181	18 (48.6%)	19 (51.4%)
182	6 (35.3%)	11 (64.7%)
191	3 (37.5%)	5 (62.5%)
193	1 (33.3%)	2 (66.7%)
194	2 (33.3%)	4 (66.7%)
195	11 (42.3%)	15 (57.7%)
196	16 (48.5%)	17 (51.5%)

Table S8. Baseline data for the assessment of (1) those in the run-in vs. randomized, (2) those termed during the run-in due to unacceptable sputum vs. termed for other reasons, and (3) those who completed vs. withdrew from the trial

Characteristic[†]	Not Randomized		Randomized		
	Other reason for Run-in Termination (N=185)	Termed due to Unacceptable Sputum (N=84)	Randomized (N=295)	Completers (N=241)	Dropouts (N=54)
Demographics					
Age at enrollment	29.3±12.8	26.9±13.7	31.2±13.9	31.2±14.4	31.0±11.7
Male – no. of participants (%)	63 (34.1%)	23 (27.4%)	111 (37.6%)	92 (38.2%)	19 (35.2%)
Race/Ethnicity – no. of participants (%):					
AI/AN	2 (1.1%)	0 (0.0%)	0 (0.0%)		
Asian/PI	5 (2.7%)	2 (2.4%)	11 (3.7%)	9 (3.7%)	2 (3.7%)
Black	79 (42.7%)	31 (36.9%)	88 (29.8%)	66 (27.4%)	22 (40.7%)
White	73 (39.5%)	37 (44.0%)	168 (56.9%)	143 (59.3%)	25 (46.3%)
Hispanic	22 (11.9%)	13 (15.5%)	24 (8.1%)	19 (7.9%)	5 (9.3%)
Other	4 (2.2%)	1 (1.2%)	4 (1.4%)	4 (1.7%)	0 (0.0%)
Asthma History					
Median Age when doctor first diagnosed (interquartile range)	6.0 (2.0-14.0)	6.0 (3.0-18.0)	8.0 (3.0-14.0)	9.0 (3.0-15.0)	6.5 (3.0-12.0)
Duration of asthma (years since doctor first diagnosed)	18.8±10.8	15.0±9.1	19.4±11.2	19.1±11.3	20.8±10.7
Family History of Asthma - no. of participants (%)	132 (74.2%)	55 (67.1%)	193 (68.4%)	152 (66.4%)	41 (77.4%)
Prior Year – no. of participants (%):					
One or more asthma episodes requiring emergency care or unscheduled office visit	59 (31.9%)	22 (26.5%)	69 (23.5%)	56 (23.3%)	13 (24.1%)
One or more overnight hospitalizations due to asthma	5 (2.7%)	0 (0.0%)	7 (2.4%)	5 (2.1%)	2 (3.7%)
One or more courses of systemic corticosteroid therapy taken for asthma	40 (21.6%)	15 (18.1%)	55 (18.7%)	43 (17.9%)	12 (22.2%)

Characteristic†	Not Randomized		Randomized		
	Other reason for Run-in Termination (N=185)	Termed due to Unacceptable Sputum (N=84)	Randomized (N=295)	Completers (N=241)	Dropouts (N=54)
Asthma History					
Days of work, school, or housework missed due to asthma:					
0 days	107 (57.8%)	55 (66.3%)	189 (64.5%)	154 (64.2%)	35 (66.0%)
1 to 7 days	57 (30.8%)	17 (20.5%)	79 (27.0%)	65 (27.1%)	14 (26.4%)
> 7 days	21 (11.4%)	11 (13.3%)	25 (8.5%)	21 (8.8%)	4 (7.5%)
ICS (not including combination meds)	38 (20.8%)	24 (28.9%)	61 (20.7%)	50 (20.8%)	11 (20.4%)
ICS/LABA Combination Therapy	16 (8.7%)	12 (14.5%)	29 (9.9%)	23 (9.6%)	6 (11.3%)
Inhaled Muscarinic Antagonist	3 (1.6%)	1 (1.2%)	5 (1.7%)	4 (1.7%)	1 (1.9%)
LTRA / 5LO Inhibitors	15 (8.1%)	19 (22.6%)	31 (10.5%)	26 (10.8%)	5 (9.3%)
Clinical and spirometric features					
BMI at enrollment (kg/m^2)	29.0±8.8	26.3±7.6	28.5±7.4	28.4±7.3	29.0±8.2
FEV_1 % predicted at randomization			91.9±12.1	92.2±12.0	90.3±12.4
FEV_1/FVC ratio at randomization			0.76±0.08	0.76±0.08	0.77±0.09
PC_{20} (mg/ml) at enrollment – geometric mean ± CV	1.38 (1.43)	2.28 (1.38)	2.08 (1.30)	2.07 (1.30)	2.10 (1.33)
Bronchodilator Response (4 puffs) at enrollment (relative % change)	11.2±8.6	10.5±8.2	10.4±7.6	10.0±7.3	12.3±8.4
Median eNO (ppb) at enrollment (interquartile range)	25.5 (14.0-61.0)	28.0 (16.0-53.0)	25.5 (16.0-50.0)	25.0 (16.0-50.0)	26.0 (14.0-52.0)
Median Blood Eosinophils (%) at enrollment (interquartile range)	3.2 (1.9-6.0)	3.0 (1.8-5.6)	3.0 (1.7-5.0)	3.0 (1.7-5.0)	3.0 (1.7-5.0)
Median Periostin (ng/mL) at enrollment (interquartile range)	54.6 (45.5-66.2)	61.0 (48.0-73.6)	53.2 (44.5-66.4)	53.5 (43.6-68.0)	50.1 (44.8-63.4)
Median ACT Score at randomization[1] (interquartile range)			21.0 (19.0-23.0)	21.0 (20.0-23.0)	21.0 (19.0-23.0)

† Means ± SD presented unless otherwise noted.

1 Individual ACT questions are scaled 1 to 5, with higher values representing better asthma control. ACT score is sum of questions 1-5.